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(54) Title: AMINO-SUBSTITUTED PYRIMIDINES, DERIVATIVES AND METHODS OF USE THEREFOR

(57) Abstract

The present invention relates to compositions and methods for inhibiting nonenzymatic cross-linking (protein aging). Accordingly, a composition is disclosed which comprises an agent capable of inhibiting the formation of advanced glycosylation end products of target proteins by reacting with the carbonyl moiety of the early glycosylation product of such target proteins formed by their initial glycosylation. The method comprises contacting the target protein with the composition. Both industrial and therapeutic applications for the invention are envisioned, as food spoilage and animal protein aging can be treated.

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AMINO-SUBSTITUTED PYRIMIDINES, DERIVATIVES
AND METHODS OF USE THEREFOR

RELATED PUBLICATIONS

5

The Applicants are co-authors of the following articles directed to the subject matter of the present invention: "COVALENT ATTACHMENT OF SOLUBLE PROTEINS BY NONENZYMATIALLY GLYCOSYLATED COLLAGEN: ROLE IN THE IN SITU FORMATION OF IMMUNE COMPLEXES", Brownlee et al., J. Exp. Med., 158, pp. 1730-1744 (1983); and "AGING OF PROTEINS: ISOLATION AND IDENTIFICATION OF FLUORESCENT CHROMOPHORE FROM THE REACTION OF POLYPEPTIDES WITH GLUCOSE", Pongor et al., Proc. Natl. Acad. Sci. USA, 81, pp. 2684-2688, (1984), and "ADVANCED GLYCOSYLATION ENDPRODUCTS IN TISSUE AND THE BIOCHEMICAL BASIS OF DIABETIC COMPLICATIONS", Brownlee et al., The New Eng. J. of Med., 318, pp. 1315-1321 (1988). All of the above publications are incorporated herein by reference.

20

BACKGROUND OF THE INVENTION

The present invention relates generally to the aging of proteins resulting from their reaction with glucose and other reducing sugars, and more particularly to the inhibition of the reaction of nonenzymatically glycosylated proteins and the often resultant formation of advanced glycosylation endproducts and cross-links.

30 The reaction between glucose and proteins has been known for some time. Its earliest manifestation was in the appearance of brown pigments during the cooking of food, which was identified by Maillard in 1912, who observed that glucose or other reducing sugars react with amino acids to form adducts that undergo a series of dehydrations and rearrangements to form stable brown pigments. Maillard, C.R. Acad. Sci., 154, pp. 66-68, (1912). Further studies have suggested that stored and

heat treated foods undergo nonenzymatic browning as a result of the reaction between glucose and the polypeptide chain, and that the proteins are resultingly cross-linked and correspondingly exhibit decreased 5 bioavailability.

This reaction between reducing sugars and food proteins was found to have its parallel in vivo. Thus, the nonenzymatic reaction between glucose and the free amino 10 groups on proteins to form a stable, 1-deoxyketosyl adduct, known as the Amadori product, has been shown to occur with hemoglobin, wherein a rearrangement of the amino terminal of the beta-chain of hemoglobin by reaction with glucose, forms the adduct known as 15 hemoglobin A_{1c}. The reaction has also been found to occur with a variety of other body proteins, such as lens crystallins, collagen and nerve proteins. See, Bunn et al., Biochem. Biophys. Res. Comm., 67, pp. 103-109 20 (1975); Koenig et al., J. Biol. Chem., 252, pp. 2992-2997 (1977); Monnier et al., in Maillard Reaction in Food and Nutrition, ed. Waller, G.A., American Chemical Society, 215, pp.431-448 (1983); and Monnier and Cerami, Clinics in Endocrinology and Metabolism, 11, pp. 431-452 (1982). 25 Moreover, brown pigments with spectral and fluorescent properties similar to those of late-stage Maillard products have also been observed in vivo in association with several long-lived proteins, such as lens proteins and collagen from aged individuals. An age-related 30 linear increase in pigment was observed in human dura collagen between the ages of 20 to 90 years. See, Monnier et al., Science, 211, pp. 491-493 (1981); Monnier et al., Biochem. Biophys. Acta, 760, pp. 97-103 (1983); and, Monnier et al., Proc. Nat. Acad. Sci., 81, pp. 583- 35 587 (1984). Interestingly, the aging of collagen can be mimicked in vitro by the cross-linking induced by glucose; and the capture of other proteins and the

formation of adducts by collagen, also noted, is theorized to occur by a cross-linking reaction, and is believed to account for the observed accumulation of albumin and antibodies in kidney basement membrane. See,
5 Brownlee et al, J. Exp. Med., 158, pp. 1739-1744 (1983); and Kohn et al., Diabetes, 33, No. 1, pp. 57-59 (1984).

In Parent Application Serial No. 798,032, a method and associated agents were disclosed that served to inhibit
10 the formation of advanced glycosylation endproducts by reacting with the early glycosylation product that results from the original reaction between the target protein and glucose. Accordingly, inhibition was postulated to take place as the reaction between the
15 inhibitor and the early glycosylation product appeared to interrupt the subsequent reaction of the glycosylated protein with additional protein material to form the cross-linked late stage product. One of the agents identified as an inhibitor was aminoguanidine, and the
20 results of further testing have borne out its efficacy in this regard.

While the success that has been achieved with aminoguanidine and similar compounds is promising, a need
25 continues to exist to identify and develop additional inhibitors that broaden the availability and perhaps the scope of this potential activity and its diagnostic and therapeutic utility.

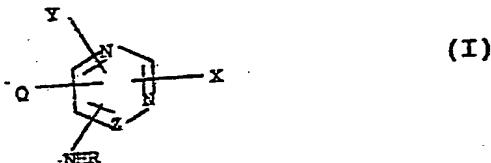
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SUMMARY OF THE INVENTION

In accordance with the present invention, a method and compositions are disclosed for the inhibition of the advanced glycosylation of proteins (protein aging). In
35 particular, the compositions comprise agents for inhibiting nonenzymatic cross-linking (protein aging) due to the formation of advanced glycosylation endproducts.

The agents may be selected from those materials capable of reacting with the early glycosylation product from the reaction of glucose with proteins and preventing further reactions. Cross-linking caused by other reactive sugars 5 present in vivo or in foodstuffs, including ribose, galactose and fructose would also be prevented by the methods and compositions of the present invention.

The agents comprise compounds having the following 10 structural formula:



15 wherein Z is N or CH-; X, Y and Q are each independently a hydrogen, amino, heterocyclo, amino lower alkyl, lower alkyl, thio or hydroxy group; 20 and R is hydrogen or an amino group; and their corresponding 3-oxides; and their biocompatible and pharmaceutically acceptable salts and mixtures thereof, and a carrier therefor.

25 The compounds utilized in the compositions of this invention appear to react with the early glycosylation product thereby preventing the same from later forming the advanced glycosylation end products which lead to protein cross-links, and thereby, to protein aging.

30 The present invention also relates to a method for inhibiting protein aging by contacting the initially glycosylated protein at the stage of the early glycosylation product with a quantity of one or more of 35 the agents of the present invention, or a composition containing the same. In the instance where the present method has industrial application, one or more of the

agents may be applied to the proteins in question, either by introduction into a mixture of the same in the instance of a protein extract, or by application or introduction into foodstuffs containing the protein or 5 proteins, all to prevent premature aging and spoilage of the particular foodstuffs.

The ability to inhibit the formation of advanced glycosylation endproducts carries with it significant 10 implications in all applications where protein aging is a serious detriment. Thus, in the area of food technology, the retardation of food spoilage would confer an obvious economic and social benefit by making certain foods of marginal stability less perishable and therefore more 15 available for consumers. Spoilage would be reduced as would the expense of inspection, removal, and replacement, and the extended availability of the foods could aid in stabilizing their price in the marketplace. Similarly, in other industrial applications where the 20 perishability of proteins is a problem, the admixture of the agents of the present invention in compositions containing such proteins would facilitate the extended useful life of the same. Presently used food preservatives and discoloration preventatives such as 25 sulfur dioxide, known to cause toxicity including allergy and asthma in animals, can be replaced with compounds such as those described herein.

The present method has particular therapeutic application 30 as the Maillard process acutely affects several of the significant protein masses in the body, among them collagen, elastin, lens proteins, and the kidney glomerular basement membranes. These proteins deteriorate both with age (hence the application of the 35 term "protein aging") and a consequence of diabetes. Accordingly, the ability to either retard or substantially inhibit the formation of advanced

glycosylation endproducts carries the promise of treatment for diabetes and of course, improving the quality and, perhaps, duration of animal life.

- 5 The present agents are also useful in the area of personal appearance and hygiene, as they prevent the staining of teeth by cationic anti-microbial agents with anti-plaque properties, such as chlorhexidine.
- 10 Accordingly, it is a principal object of the present invention to provide a method for inhibiting the extensive cross-linking of proteins that occurs as an ultimate consequence of the reaction of the proteins with glucose and other reactive sugars, by correspondingly inhibiting the formation of advanced glycosylation endproducts.

It is a further object of the present invention to provide a method as aforesaid which is characterized by a reaction with an initially glycosylated protein identified as an early glycosylation product.

It is a further object of the present invention to provide a method as aforesaid which prevents the rearrangement and cross-linking of the said early glycosylation products to form the said advanced glycosylation endproducts.

It is a yet further object of the present invention to provide agents capable of participating in the reaction with the said early glycosylation products in the method as aforesaid.

It is a still further object of the present invention to provide therapeutic methods of treating the adverse consequences of protein aging by resort to the aforesaid method and agents.

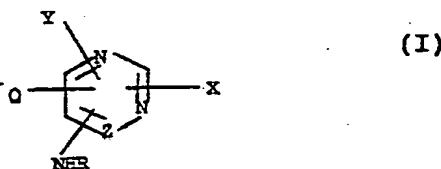
It is a still further object of the present invention to provide a method of inhibiting the discoloration of teeth by resort to the aforesaid method and agents.

5 It is a still further object of the present invention to provide compositions including pharmaceutical compositions, all incorporating the agents of the present invention.

10 Other objects and advantages will become apparent to those skilled in the art from a consideration of the ensuing description.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

15 In accordance with the present invention, agents, compositions including pharmaceutical compositions containing said agents and associated methods have been developed which are believed to inhibit the formation of 20 advanced glycosylation endproducts in a number of target proteins existing in both animals and plant material. In particular, the invention relates to a composition which may contain one or more agents comprising compounds having the structural formula

25


30 wherein Z is N or CH-;

X, Y and Q are each independently a hydrogen, amino, heterocyclo, amino lower alkyl, lower alkyl, thio or hydroxy group;

and R is hydrogen or an amino group;

35 and their corresponding 3-oxides; and their biocompatible and pharmaceutically acceptable salts and mixtures thereof, and a carrier therefor.

The compounds of Formula I wherein the X, Y or Q substituent is on a nitrogen of the ring exist as tautomers, i.e., 2-hydroxypyrimidine can exist also as 2(1H)-pyrimidine. Both forms are intended to be 5 encompassed by this invention.

The lower alkyl groups referred to herein contain 1-6 carbon atoms and include methyl, ethyl, propyl, butyl, pentyl, hexyl, and the corresponding branched chain 10 isomers thereof. The heterocyclic groups referred to herein contain from 3-6 carbon atoms and are exemplified by groups such as pyrrolidinyl, 2-methylpyrrolidinyl, piperidinol, 2-methylpiperidino morpholino, and hexamethyleneamino.

15 The "floating" X, Y, Q and NHR bonds in Formula I indicate that these variants can be attached to the ring structure at any available carbon juncture. The hydroxy variant of X, Y and Q can also be present on a nitrogen 20 atom.

Of the compounds encompassed by Formula I, certain combinations of substituents are preferred. For instance, compounds having R as hydrogen, as a CH group, 25 and at least one of X, Y or Q as another amino group, are preferred. The group of compounds where R is hydrogen, Z is a CH group and one of X or Y is an amino lower alkyl group are also preferred. Another preferred group of compounds is those where R is hydrogen and Z is 30 N (nitrogen). Certain substitution patterns are preferred, i.e., the 6-position (IUPAC numbering, Z=CH) is preferably substituted, and most preferably by an amino or a nitro containing group. Also preferred are compounds where two or more of X, Y and Q are other than 35 hydrogen.

Representative compounds of the present invention are:

2-hydrazino-4-hydroxy-6-methylpyrimidine;
4,5-diaminopyrimidine;
4-amino-5-aminomethyl-2-methylpyrimidine;
5 6-(piperidino)-2,4-diaminopyrimidine 3-oxide;
3-amino-6-methyl-1,2,4-triazin-5(2H)-one;
4,6-diaminopyrimidine;
4,5,6-triaminopyrimidine;
4,5-diamino-6-hydroxypyrimidine;
10 2,4,5-triamino-6-hydroxypyrimidine;
5,6-diamino-2,4-dihydroxypyrimidine;
2,4,6-triaminopyrimidine;
4,5-diamino-2-methylpyrimidine;
4,5-diamino-2,6-dimethylpyrimidine;
15 4,5-diamino-2-hydroxy-pyrimidine;
4,5-diamino-2-hydroxy-6-methylpyrimidine;
2-hydrazinopyrimidine;
4,6-dimethyl-2-hydrazinopyrimidine;
3-hydrazino-1,2,4-triazine;
20 3-hydrazino-5-hydroxy-1,2,4-triazine;
5-hydrazino-3-hydroxy-1,2,4-triazine;
5,6-diamino-3-hydroxy-1,2,4-triazine;
6-methyl-2,4,5-triaminopyridine;
2,4,5,6-tetraaminopyridine;
25 4,5-diamino-2,6-dithiopyrimidine;
4,5-diamino-2-thiopyrimidine;
4,5-diamino-6-hydroxy-2-thiopyrimidine;
4,5-diamino-2-hydroxy-6-thiopyrimidine; and
4,5-diamino-6-methyl-2-thiopyrimidine.
30
The above compounds are capable of inhibiting the formation of advanced glycosylation endproducts on target proteins. The cross-linking of the protein to form the advanced glycosylation endproduct contributes to the 35 entrapment of other proteins and results in the development in vivo of conditions such as reduced elasticity and wrinkling of the skin, certain kidney

diseases, atherosclerosis, osteoarthritis and the like. Similarly, plant material that undergoes nonenzymatic browning deteriorates and, in the case of foodstuffs, become spoiled or toughened and, consequently, inedible.

5 Thus, the compounds employed in accordance with this invention inhibit this late stage Maillard effect and intervene in the deleterious changes described above.

The rationale of the present invention is to use agents 10 which block the post-glycosylation step, i.e., the formation of fluorescent chromophores such as that identified in Pongor, et al., supra and Farmar et al., supra, among others, the presence of which chromophores is associated with, and leads to adverse sequelae of 15 diabetes and aging. An ideal agent would prevent the formation of the chromophore and its associate cross-links of proteins to proteins and trapping of proteins on the other proteins, such as occurs in arteries and in the kidney.

20 The chemical nature of the early glycosylation products with which the compounds of the present invention are believed to react, is speculative. Early glycosylation products with carbonyl moieties that are involved in the 25 formation of advanced glycosylation endproducts, and that may be blocked by reaction with the compounds of the present invention, have been postulated. In one case, the reactive carbonyl moieties of Amadori products or their further condensation, dehydration and/or 30 rearrangement products, may condense to form advanced glycosylation endproducts. Another proposed mechanism is the formation of reactive carbonyl compounds, containing one or more carbonyl moieties (such as glycolaldehyde, glyceraldehyde or 3-deoxyglucosone) from the cleavage of 35 Amadori or other early glycosylation endproducts (see, for example, Gottschalk, A. (1972) in *The Glycoproteins* (Gottschalk, A., ed) Part A, pp. 141-157, Elsevier

Publishing Co., New York; Reynolds, T.M. (1965) Adv. Food Res., 14, pp. 167-283), and by subsequent reactions with an amine or Amadori product to form carbonyl containing advanced glycosylation products such as the alkylformyl-
5 glycosylpyrroles described by Farmar et al, supra.

Several investigators have studied the mechanism of advanced glycosylation product formation. In vitro studies by Eble et al., (1983), "Nonenzymatic
10 Glucosylation and Glucose-dependent Cross-linking of Protein", J. Biol. Chem., 258:9406-9412, concerned the cross-linking of glycosylated protein with nonglycosylated protein in the absence of glucose. Eble et al. sought to elucidate the mechanism of the Maillard
15 reaction and accordingly conducted controlled initial glycosylation of RNAase as a model system, which was then examined under varying conditions. In one aspect, the glycosylated protein material was isolated and placed in a glucose-free environment and thereby observed to
20 determine the extent of cross-linking.

Eble et al. thereby observed that cross-linking continued to occur not only with the glycosylated protein but with non-glycosylated proteins as well. One of the
25 observations noted by Eble et al. was that the reaction between glycosylated protein and the protein material appeared to occur at the location on the protein chain of the amino acid lysine. Confirmatory experimentation conducted by Eble et al. in this connection demonstrated
30 that free lysine would compete with the lysine on RNAase for the binding of glycosylated protein. Thus, it might be inferred from these data that lysine may serve as an inhibitor of advanced glycosylation; however, this conclusion and the underlying observations leading to it
35 should be taken in the relatively limited context of the model system prepared and examined by Eble et al. Clearly, Eble et al. does not appreciate, nor is there a

suggestion therein, of the discoveries that underlie the present invention, with respect to the inhibition of advanced glycosylation of proteins both in vitro and in vivo.

5

The experiments of Eble et al. do not suggest the reactive cleavage product mechanism or any other mechanism in the in vivo formation of advanced glycosylation endproducts in which glucose is always present. In fact, other investigators support this mechanism to explain the formation of advanced glycosylated endproducts in vivo (see for example Hayase et al, 1989, supra; Sell and Monnier, 1989, supra; Oimomi et al., Agric. Biol. Chem., 53(6):1727-1728 (1989); and 10 Diabetes Research and Clinical Practice, 6:311-313 (1989). Accordingly, the use of lysine as an inhibitor in the Eble et al. model system has no bearing upon the utility of the compounds of the present invention in the inhibition of advanced glycosylated endproducts formation 15 in the presence of glucose in vivo, and the amelioration of complications of diabetes and aging.

The compositions useful in the present invention comprise or contain agents capable of reacting with the active 20 carbonyl intermediate of an early glycosylation product. Suitable agents are the compounds of Formula I of the 25 present invention.

The present invention likewise relates to methods for 30 inhibiting the formation of advanced glycosylation endproducts, which comprise contacting the target proteins with a composition of the present invention. In the instance where the target proteins are contained in foodstuffs, whether of plant or animal origin, these 35 foodstuffs could have applied to them by various conventional means a composition containing the present agents.

In the food industry, sulfites were found years ago to inhibit the Maillard reaction and are commonly used in processed and stored foods. Recently, however, sulfites in food have been implicated in severe and even fatal 5 reactions in asthmatics. As a consequence, the sulfite treatment of fresh fruits and vegetables has been banned. The mechanism for the allergic reaction is not known. Accordingly, the present compositions and agents offer a nontoxic alternative to sulfites in the treatment of 10 foods in this manner.

As is apparent from a discussion of the environment of the present invention, the present methods and compositions hold the promise for arresting the aging of 15 key proteins both in animals and plants, and concomitantly, conferring both economic and medical benefits as a result thereof. In the instance of foodstuffs, the administration of the present composition holds the promise for retarding food spoilage thereby 20 making foodstuffs of increased shelf life and greater availability to consumers. Replacement of currently-used preservatives, such as sulfur dioxide known to cause allergies and asthma in humans, with non-toxic, biocompatible compounds is a further advantage of the 25 present invention.

The therapeutic implications of the present invention relate to the arrest of the aging process which has, as indicated earlier, been identified in the aging of key 30 proteins by advanced glycosylation and cross-linking. Thus, body proteins, and particularly structural body proteins, such as collagen, elastin, lens proteins, nerve proteins, kidney glomerular basement membranes and other extravascular matrix components would all benefit in 35 their longevity and operation from the practice of the present invention. The present invention thus reduces the incidence of pathologies involving the entrapment of

proteins by cross-linked target proteins, such as retinopathy, cataracts, diabetic kidney disease, glomerulosclerosis, peripheral vascular disease, arteriosclerosis obliterans, peripheral neuropathy, 5 stroke, hypertension, atherosclerosis, osteoarthritis, periarticular rigidity, loss of elasticity and wrinkling of skin, stiffening of joints, glomerulonephritis, etc. Likewise, all of these conditions are in evidence in patients afflicted with diabetes mellitus. Thus, the 10 present therapeutic method is relevant to treatment of the noted conditions in patients either of advanced age or those suffering from one of the mentioned pathologies.

Protein cross-linking through advanced glycosylation product formation can decrease solubility of structural proteins such as collagen in vessel walls (see Brownlee et al., Science, 232, pp. 1629-1632, (1986)), and can also trap serum proteins, such as lipoproteins to the collagen. Also, this may result in increased 15 permeability of the endothelium and consequently covalent trapping of extravasated plasma proteins in subendothelial matrix, and reduction in susceptibility of both plasma and matrix proteins to physiologic degradation by enzymes. (See Brownlee et al., Diabetes, 20 35, Suppl. 1, p. 42A (1986)). For these reasons, the progressive occlusion of diabetic vessels induced by chronic hyperglycemia has been hypothesized to result 25 from excessive formation of glucose-derived cross-links. Such diabetic macrovascular changes and microvascular 30 occlusion can be effectively prevented by chemical inhibition of advanced glycosylation product formation utilizing a composition and the methods of the present invention.

35 Studies indicate that the development of chronic diabetic damage in target organs is primarily linked to hyperglycemia so that tight metabolic control would delay

or even prevent end-organ damage. See Nicholls et al., Lab. Invest., 60, No. 4, p. 486 (1989), which discusses the effects of islet isografting and aminoguanidine in murine diabetic nephropathy. These studies further 5 evidence that aminoguanidine diminishes aortic wall protein cross-linking in diabetic rats and confirm earlier studies by Brownlee et al., Science, 232, pp. 1629-1632 (1986) to this additional target organ of complication of diabetes. Also, an additional study 10 showed the reduction of immunoglobulin trapping in the kidney by aminoguanidine (Brownlee et al., Diabetes, 35, Suppl. 1, p. 42A (1986)).

Further evidence in the streptozotocin-diabetic rat model 15 that aminoguanidine administration intervenes in the development of diabetic nephropathy was presented by Brownlee et al., 1988, supra, with regard to morphologic changes in the kidney which are hallmarks of diabetic renal disease. These investigators reported that the 20 increased glomerular basement membrane thickness, a major structural abnormality characteristic of diabetic renal disease, was prevented with aminoguanidine.

Taken together, these data strongly suggest that 25 inhibition of the formation of advanced glycosylation endproducts (AGEs), by the teaching of the present invention, may prevent late, as well as early, structural lesions due to diabetes, as well as changes during aging caused by the formation of AGE's.

30 Diabetes-induced changes in the deformability of red blood cells, leading to more rigid cell membranes, is another manifestation of cross-linking and aminoguanidine has been shown to prevent it in vivo. In such studies, 35 New Zealand White rabbits, with induced, long-term diabetes are used to study the effects of a test compound on red blood cell (RBC) deformability (df). The test

compound is administered at a rate of 100 mg/kg by oral gavage to diabetic rabbits (Brown et al., Presentation of Abstract for Association for Academic Minority Physicians, Annual Scientific Meeting (1989)).

5 Increased cross-linking of collagen in diabetic rats has shown to be prevented by aminoguanidine. Oxlund and Andreassen, "The increase in biochemical and biomechanical stability of collagen in diabetic rats is prevented by aminoguanidine treatment", European 10 Association for the Study of Diabetes, Twenty-fifth Annual Meeting, p. 525A, Abstract No. 371, 1989 showed the effect when thermal stability of tendon fibers was assessed by breaking time in a urea bath, as well as 15 mechanical strength. Soulis et al., "Aminoguanidine reduces tissue fluorescence but not albuminuria in diabetic rats". NIH Conference on the Maillard Reaction in Aging, Diabetes, and Nutrition, Bethesda, Maryland, September 22-23, 1988, page 30) showed the same effect on 20 collagen in the aorta, measured by fluorescence and solubility.

Giambione and Brownlee, "Aminoguanidine Treatment Normalizes Increased Steady-state Levels of Laminin B1 mRNA in Kidneys of Long-term Streptozotocin-diabetic 25 Rats" Diabetes, 38, Supplement 2:83A Forty-ninth Annual Meeting, American Diabetes Association (1989) showed that aminoguanidine treatment to diabetic rats prevents the diabetes-induced increase in laminin B₁ mRNA in the 30 kidney. This indicates that aminoguanidine may prevent overproduction of matrix, which leads to basement membrane thickening and morphologic and functional deterioration of vasculature in kidneys and other organs.

35 A further consequence of diabetes is the hyperglycemia-induced matrix bone differentiation resulting in decreased bone formation usually associated with chronic

diabetes. In animal models, diabetes reduces matrix-induced bone differentiation by 70% (Am. J. Phys., 238 (1980)).

5 In the instance where the compositions of the present invention are utilized for in vivo or therapeutic purposes, it may be noted that the compounds or agents used therein are biocompatible. Pharmaceutical compositions may be prepared with a therapeutically 10 effective quantity of the agents or compounds of the present invention and may include a pharmaceutically acceptable carrier, selected from known materials utilized for this purpose. Such compositions may be prepared in a variety of forms, depending on the method 15 of administration. Also, various pharmaceutically acceptable addition salts of the compounds of Formula I may be utilized.

A liquid form would be utilized in the instance where 20 administration is by intravenous, intramuscular or intraperitoneal injection. When appropriate, solid dosage forms such as tablets, capsules, or liquid dosage formulations such as solutions and suspensions, etc., may be prepared for oral administration. For topical or 25 dermal application to the skin or eye, a solution, a lotion or ointment may be formulated with the agent in a suitable vehicle such as water, ethanol, propylene glycol, perhaps including a carrier to aid in penetration into the skin or eye. For example, a topical preparation 30 could include up to about 10% of the compound of Formula I. Other suitable forms for administration to other body tissues are also contemplated.

In the instance where the present method has therapeutic 35 application, the animal host intended for treatment may have administered to it a quantity of one or more of the agents, in a suitable pharmaceutical form.

Administration may be accomplished by known techniques, such as oral, topical and parenteral techniques such as intradermal, subcutaneous, intravenous or intraperitoneal injection, as well as by other conventional means.

5 Administration of the agents may take place over an extended period of time at a dosage level of, for example, up to about 25 mg/kg.

As noted earlier, the invention also extends to a method 10 of inhibiting the discoloration of teeth resulting from nonenzymatic browning in the oral cavity which comprises administration to a subject in need of such therapy an amount effective to inhibit the formation of advanced glycosylation endproducts of a composition comprising an 15 agent of structural Formula I.

The nonenzymatic browning reaction which occurs in the oral cavity results in the discoloration of teeth. Presently used anti-plaque agents accelerate this 20 nonenzymatic browning reaction and further the staining of the teeth. Recently, a class of cationic anti-microbial agents with remarkable anti-plaque properties have been formulated in oral rinses for regular use to kill bacteria in the mouth. These agents, the cationic 25 antiseptics, include such agents as alexidine, cetyl pyridinium chloride, chlorhexidine gluconate, hexetidine, and benzalkonium chloride.

Tooth staining by chlorhexidine and other anti-plaque 30 agents apparently results from the enhancement of the Maillard reaction. Nordbo, J. Dent. Res., 58, p. 1429 (1979) reported that chlorhexidine and benzalkonium chloride catalyze browning reactions in vitro. Chlorhexidine added to mixtures containing a sugar 35 derivative and a source of amino groups underwent increased color formation, attributed to the Maillard reaction. It is also known that use of chlorhexidine

results in an increased dental pellicle. Nordbo proposed that chlorhexidine resulted in tooth staining in two ways: first, by increasing formation of pellicle which contains more amino groups, and secondly, by catalysis of 5 the Maillard reaction leading to colored products.

In accordance with this method, the compounds of Formula I are formulated into compositions adapted for use in the oral cavity. Particularly suitable formulations are oral 10 rinses and toothpastes incorporating the active agent.

In the practice of this invention, conventional formulating techniques are utilized with nontoxic, pharmaceutically acceptable carriers typically utilized 15 in the amounts and combinations that are well-known for the formulation of such oral rinses and toothpastes.

The agent of Formula I is formulated in compositions in an amount effective to inhibit the formation of advanced 20 glycosylation endproducts. This amount will, of course, vary with the particular agent being utilized and the particular dosage form, but typically is in the range of 0.01% to 1.0%, by weight, of the particular formulation.

25 Additionally, since the agents of the aforesaid method are concentrated in the salivary glands upon oral ingestion or parenteral administration, they can be so administered. This concentration in the salivary glands results in their secretion into saliva, the net result 30 being that they are functionally placed in the oral cavity where they can effect their desired method. For such administration, the particular agent can be formulated in any conventional oral or parenteral dosage form. A particularly desirable dosage form is the 35 incorporation of the agent into a vitamin tablet or fluoride tablet so as to maximize patient, and particularly juvenile patient, compliance.

The compounds encompassed by Formula I are conveniently prepared by chemical syntheses well-known in the art. Certain of the compounds encompassed by Formula I are well-known compounds readily available from chemical supply houses and/or preparable by synthetic methods specifically published therefor. For instance, the following compounds are commercially available from Aldrich Chemical Company (Milwaukee, Wisconsin) or Sigma Chemical Company (St. Louis, Missouri):

5 2-hydrazino-4-hydroxy-6-methylpyrimidine;

10 4,5-diaminopyrimidine;

4-amino-5-aminomethyl-2-methylpyrimidine;

6-(1-piperidino)-2,4-diaminopyrimidine 3-oxide;

3-amino-6-methyl-1,2,4-triazin-5(2H)-one;

15 4,6-diaminopyrimidine;

4,5,6-triaminopyrimidine;

4,5-diamino-6-hydroxypyrimidine;

2,4,5-triamino-6-hydroxypyrimidine;

5,6-diamino-2,4-dihydroxypyrimidine;

20 2,4,6-triaminopyrimidine;

6-methyl-2,4,5-triaminopyridine sulfate;

2,4,5,6-tetraaminopyridine sulfate;

4,5-diamino-2,6-dithiopyrimidine;

4,5-diamino-2-thiopyrimidine;

25 4,5-diamino-6-hydroxy-2-thiopyrimidine;

4,5-diamino-2-hydroxy-6-thiopyrimidine hemisulfate; and

4,5-diamino-6-methyl-2-thiopyrimidine.

30 Other compounds described in the chemical and patent literature or directly preparable by methods described therein and encompassed by Formula I are those such as

4,5-diamino-2-methylpyrimidine;

4,5-diamino-2,6-dimethylpyrimidine;

4,5-diamino-2-hydroxypyrimidine;

35 4,5-diamino-2-hydroxy-6-methylpyrimidine;

2-hydrazinopyrimidine;

3-hydrazino-1,2,4-triazine;

3-hydrazino-5-hydroxy-1,2,4-triazine;
3-hydrazino-3-hydroxy-1,2,4-triazine;
5,6-diamino-3-hydroxy-1,2,4-triazine;

5 and their pharmaceutically acceptable acid or alkali addition salts.

EXAMPLE I

10 The following method was used to evaluate the ability of the compounds of the present invention to inhibit glucose-mediated development of fluorescence of bovine serum albumin (BSA), a measure of cross-linking. Compounds were incubated under aseptic conditions at a 15 concentration of 1 mM with 400 mM glucose and 100 mg/mL BSA in a 1.5 M sodium phosphate buffer, pH 7.4.

Samples of the incubation mixture were taken immediately and after 1 week incubation at 37°C for measurement of 20 fluorescence. For each test compound, control incubations in buffer were made of compound alone (C), compound plus glucose (G+C), and compound plus BSA (B+C). An additional set of incubations of glucose and BSA (B+G) were prepared as the baseline controls against which were 25 measured the ability of the compounds to inhibit. Each incubation was made in triplicate.

Fluorescence (excitation, 370 nm; emission, 440 nm) was measured on each sample after a 100-fold dilution in 30 distilled water.

The % inhibition of browning of each test compound was calculated as follows. Each ΔF represents the fluorescence measurement of that sample after 1 week 35 incubation less its fluorescence before incubation.

$$\% \text{ inhibition} = \frac{F_{B+G} - [F_{B+G+C} - (F_B + F_G + F_C)] \times 100}{F_{B+G}}$$

5

where B=BSA, G=glucose, and C=test compound.

Percent inhibition of browning by various test compounds at 1 mM:

10	0%	no inhibitor;
	35.1%	4,5-diaminopyrimidine;
	47.7%	4-amino-5-aminomethyl-2-methylpyrimidine;
	37.1%	6-(1-piperidino)-2,4-diaminopyrimidine 3-oxide;
	68.9%	4,5-diamino-2-thiopyrimidine; and
15	79.2%	4,5-diamino-6-methyl-2-thiopyridine.

The above experiments suggest that this type of drug therapy may have benefit in reducing the pathology associated with the advanced glycosylation of proteins and the formation of cross-links between proteins and other macromolecules. Drug therapy may be used to prevent the increased trapping and cross-linking of proteins that occurs in diabetes and aging which leads to sequelae such as retinal damage, and extra-vascularly, damage to tendons, ligaments and other joints. This therapy might retard atherosclerosis and connective tissue changes that occur with diabetes and aging. Both topical, oral, and parenteral routes of administration to provide therapy locally and systemically are contemplated.

EXAMPLE 2

	<u>Tablet</u>	<u>mg/tablet</u>
35	Compound of Formula I	50
	Starch	50
	Mannitol	75

Magnesium stearate	2
Stearic acid	5

The compound, a portion of the starch and the lactose are
 5 combined and wet granulated with starch paste. The wet
 granulation is placed on trays and allowed to dry
 overnight at a temperature of 45°C. The dried
 granulation is comminuted in a comminutor to a particle
 size of approximately 20 mesh. Magnesium stearate,
 10 stearic acid and the balance of the starch are added and
 the entire mix blended prior to compression on a suitable
 tablet press. The tablets are compressed at a weight of
 232 mg. using a 11/32" punch with a hardness of 4 kg.
 These tablets will disintegrate within a half hour
 15 according to the method described in USP XVI.

EXAMPLE 3

	<u>Lotion</u>	<u>mg/g</u>
20	Compound of Formula I	1.0
	Ethyl alcohol	400.0
	Polyethylene glycol 400	300.0
	Hydroxypropyl cellulose	5.0
	Propylene glycol	to make 1.0 g

25

EXAMPLE 4

	<u>Oral Rinse</u>	
	Compound of Formula I:	1.4 %
30	Chlorhexidine gluconate	0.12 %
	Ethanol	11.6 %
	Sodium saccharin	0.15 %
	FD&C Blue No. 1	0.001%
	Peppermint Oil	0.5 %
35	Glycerine	10.0 %
	Tween 60	0.3 %
	Water to	100 %

EXAMPLE 5

<u>Toothpaste</u>		
Compound of Formula I:		5.5 %
5 Sorbitol, 70% in water		25 %
Sodium saccharin		0.15 %
Sodium lauryl sulfate		1.75 %
Carbopol 934, 6% dispersion in water		15 %
Oil of Spearmint		1.0 %
10 Sodium hydroxide, 50% in water		0.76 %
Dibasic calcium phosphate dihydrate		45 %
Water to		100 %

EXAMPLE 6

15 To further study the ability of inhibitors of nonenzymatic browning to prevent the discoloration of protein on a surface, such as that which occurs on the tooth surface, the following surface browning experiment
20 is performed. As a substitute for a pellicle-covered tooth surface, unexposed and developed photographic paper is used to provide a fixed protein (gelatin, i.e., collagen) surface on a paper backing. Five millimeter circles are punched and immersed for one week at 50°C in
25 a solution of 100 mM glucose-6-phosphate in a 0.5 M phosphate buffer, pH 7.4, containing 3 mM sodium azide. Glucose-6-phosphate is a sugar capable of participating in nonenzymatic browning at a more rapid rate than glucose. In addition to the glucose-6-phosphate,
30 chlorhexidine and/or a compound of Formula I are included. After incubation, the gelatin/paper disks are rinsed with water, observed for brown color, and photographed.

35 Incubation of the disks in glucose-6-phosphate alone shows slight brown color versus disks soaked in buffer alone. Inclusion of chlorhexidine (in the form of

Peridex® at a final concentration of 0.04% chlorhexidine) shows significant browning. Addition of a compound of Formula I to the chlorhexidine completely inhibits browning of the gelatin, as does inclusion of a compound 5 of Formula I in the absence of chlorhexidine.

The slight brown color formed by the action of glucose-6-phosphate on the gelatin surface alone and its prevention by a compound of Formula I demonstrates the utility of 10 the present invention in preventing nonenzymatic browning of tooth surfaces. The enhanced browning in the presence of chlorhexidine and its prevention with a compound of Formula I demonstrates the utility of the present invention in preventing the anti-plaque agent-enhanced 15 nonenzymatic browning which occurs with chlorhexidine.

This invention may be embodied in other forms or carried out in other ways without departing from the spirit or essential characteristics thereof. The present 20 disclosure is therefore to be considered as in all respects illustrative and not restrictive, the scope of the invention being indicated by the appended Claims, and all changes which come within the meaning and range of equivalency are intended to be embraced therein.

WHAT IS CLAIMED IS:

1 1. A composition for inhibiting the advanced
2 glycosylation of a target protein comprising an effective
3 amount of a compound selected from the group consisting
4 of compounds of the formula



11 X, Y and Q are each independently a hydrogen,
12 amino, heterocyclo, amino lower alkyl, lower alkyl, thio
13 or hydroxy group;
14 and R is hydrogen or an amino group;
15 and their corresponding 3-oxides; and their
16 biocompatible and pharmaceutically acceptable salts and
17 mixtures thereof, and a carrier therefor.

1 2. A pharmaceutical composition for administration to
2 an animal to inhibit the advanced glycosylation of a
3 target protein within said animal, comprising a
4 pharmaceutically effective amount of a compound selected
5 from the group consisting of compounds of the formula



12 X, Y and Q are each independently a hydrogen,
13 amino, heterocyclo, amino lower alkyl, lower alkyl, thio
14 or hydroxy group;
15 and R is hydrogen or an amino group;

16 and their corresponding 3-oxides; and their
17 biocompatible and pharmaceutically acceptable salts and
18 mixtures thereof, and a carrier therefor.

1 3. The composition of Claims 1 or 2 wherein said
2 compound has the formula wherein R is an amino group and
3 Z is a CH group.

1 4. The composition of Claims 1 or 2 wherein said
2 compound is 2-hydrazino-4-hydroxy-6-methylpyrimidine.

1 5. The composition of Claims 1 or 2 wherein said
2 compound has the formula wherein R is hydrogen, and Z is
3 a CH group and one of X, Y or Q is an amino group.

1 6. The composition of Claims 1 or 2 wherein said
2 compound is 4,5-diaminopyrimidine.

1 7. The composition of Claims 1 or 2 wherein said
2 compound is 6-(1-piperidino)-2,4-diaminopyrimidine 3-
3 oxide.

1 8. The composition of Claims 1 or 2 wherein said
2 compound is 4,5-diamino-2-thiopyridine.

1 9. The composition of Claims 1 or 2 wherein said
2 compound is 4,5-diamino-6-methyl-2-thiopyrimidine.

1 10. The composition of Claims 1 or 2 wherein said
2 compound has the formula wherein R is hydrogen, Z is a CH
3 group and one of X, Y, or Z is an amino lower alkyl group.

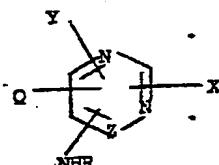
1 11. The composition of Claims 1 or 2 wherein said
2 compound is 4-amino-5-aminomethyl-2-methylpyrimidine.

1 12. The composition of Claims 1 or 2 wherein said
2 compound has the formula wherein R is hydrogen and Z is
3 N.

1 13. The composition of Claims 1 or 2 wherein said
2 compound is 3-amino-6-methyl-1,2,4-triazin-5(2H)-one.

1 14. A method for inhibiting the advanced glycosylation
2 of a target protein comprising contacting the target
3 protein with an effective amount of composition
4 comprising a compound selected from the group consisting
5 of compounds of the formula

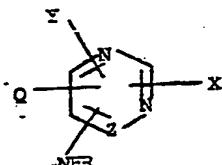
6
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11 wherein Z is N or CH-;
12 X, Y and Q are each independently a hydrogen,
13 amino, heterocyclo, amino lower alkyl, lower alkyl, thio
14 or hydroxy group;
15 and R is hydrogen or an amino group;
16 and their corresponding 3-oxides; and their
17 biocompatible and pharmaceutically acceptable salts and
18 mixtures thereof, and a carrier therefor.

1 15. A method for treating an animal to inhibit the
2 formation of advanced glycosylation endproducts of a
3 target protein within said animal, said method comprising
4 administering an effective amount of a pharmaceutical
5 composition, said pharmaceutical composition comprising a
6 compound selected from the group consisting of compounds
7 of the formula

8
9
10
11



12 wherein Z is N or CH-;
13 X, Y and Q are each independently a hydrogen,
14 amino, heterocyclo, amino lower alkyl, lower alkyl, thio or
15 hydroxy group;
16 and R is hydrogen or an amino group;
17 and their corresponding 3-oxides; and their
18 biocompatible and pharmaceutically acceptable salts and
19 mixtures thereof, and a carrier therefor.

1 16. The method of Claims 14 or 16 wherein said compound
2 has the formula wherein R is an amino group and Z is a CH
3 group.

1 17. The method of Claims 14 or 16 wherein said compound
2 is 2-hydrazino-4-hydroxy-6-methylpyrimidine.

1 18. The method of Claims 14 or 16 wherein said compound
2 has the formula wherein R is hydrogen, Z is a CH group
3 and one of X or Y is an amino group.

1 19. The method of Claims 14 or 16 wherein said compound
2 is 4,5-diaminopyrimidine.

1 20. The method of Claims 14 or 16 wherein said compound
2 is 6-(1-piperidino)-2,4-diaminopyrimidine 3-oxide.

1 21. The method of Claims 14 or 16 wherein said compound
2 is 4,5-diamino-2-thiopyrimidine.

1 22. The method of Claims 14 or 16 wherein said compound
2 is 4,5-diamino-6-methyl-2-thiopyrimidine.

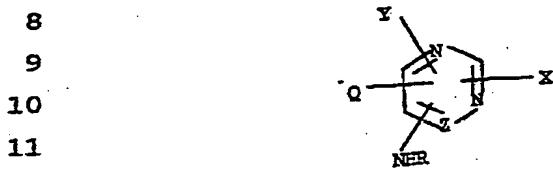
1 23. The method of Claims 14 or 16 wherein said compound
2 has the formula wherein R is hydrogen, Z is a CH group
3 and one of X or Y is an amino lower alkyl group.

1 24. The method of Claims 14 or 16 wherein said compound
2 is 4-amino-5-aminomethyl-2-methylpyrimidine.

1 25. The method of Claims 14 or 16 13 wherein said
2 compound has the formula wherein R is hydrogen and Z is
3 N.

1 26. The method of Claims 14 or 16 wherein said compound
2 is 3-amino-6-methyl-1,2,4-triazin-5(2H)-one.

1 27. A method of inhibiting the discoloration of teeth
2 resulting from non-enzymatic browning in the oral cavity
3 which comprises administration of an amount effective to
4 inhibit the formation of advanced glycosylation
5 endproducts of a composition comprising a compound
6 selected from the group consisting of compounds of the
7 formula



INTERNATIONAL SEARCH REPORT

International Application N

PCT/US 92/00152

1. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)

to International Patent Classification (IPC) or to both National Classification and IPC

According to International Patent Classification (IPC) or to the European Patent Classification

Int.C1.5 A 61 K 31/505 A 61 K 31/53 A 61 K 31/10

II. FIELDS SEARCHED

Minimum Documentation Searched

Classification System	Classification Symbols
Int.C1.5	A 61 K

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched⁸

III DOCUMENTS CONSIDERED TO BE RELEVANT⁹

Category ^a	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	WO,A,9006117 (THE UPJOHN CO.) 14 June 1990, see the whole document ---	1-2,5,7
X	The Journal of Investigation Dermatology, vol. 81, no. 6, December 1983, H.P. BADEN et al.: "Effect of minoxidil on cultured keratinocytes", pages 558-560, see the whole document ---	1-2,5,7
X	The Journal of Biological Chemistry, vol. 262, no. 25, 5 September 1987, S. MURAD et al.: "Suppression of fibroblast proliferation and lysyl hydroxylase activity by minoxidil", pages 11973-11978 ---	1-2,5,7
X	EP,A,0330263 (MERCK & CO., INC.) 30 August 1989, see abstract; example 9; claims 1,11,12 ---	1-2,5
		-/-

° Special categories of cited documents :¹⁰

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"a" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search 13-04-1992	Date of Mailing of this International Search Report 10.06.92
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International Searching Authority

EUROPEAN PATENT OFFICE

Signature of Authorized Officer

MISSAT. MORTENSEN

III. DOCUMENTS CONSIDERED TO BE RELEVANT		(CONTINUED FROM THE SECOND SHEET)
Category	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	---	14, 15, 18
Y	Science, vol. 232, 27 June 1986, M. BROWNLEE et al.: "Aminoguanidine prevents diabetes-induced arterial wall protein cross-linking", pages 1629-1632, see the whole document (cited in the application) ---	14, 15, 18
Y	The New England Journal of Medicine, vol. 318, no. 20, 1988, H. BROWNLEE et al.: "Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications", pages 1315-1321, see the whole document (cited in the application) ---	14, 15, 18
A	EP,A,0316852 (THE ROCKEFELLER UNIVERSITY) 24 May 1989, see the abstract; example 6, lines 46-50; claims 1, 79, 80 ---	1-26
A	EP,A,0222313 (THE ROCKEFELLER UNIVERSITY) 20 May 1987, see the abstract; claims ---	1-26
A	EP,A,0359112 (TAKEDA CHEMICAL INDUSTRIES), 21 March 1990, see the abstract; example 28; claims ---	1-26
A	EP,A,0339496 (ONO PHARMACEUTICAL CO.) 2 November 1989, see the abstract; table 3, example 2d; claims ---	1-26
A	EP,A,0327919 (THE ROCKEFELLER UNIVERSITY) 16 August 1989, see the abstract; example 11, lines 29-34; claims ---	27

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. OBSERVATION WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE¹ see annex

This International search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claim numbers see remark 1) because they relate to subject matter not required to be searched by this Authority, namely:

2. Claim numbers see remark 2) 3) because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically:

3. Claim numbers the second and third sentences of PCT Rule 6.4(a). because they are dependent claims and are not drafted in accordance with

VI. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING²

This International Searching Authority found multiple inventions in this International application as follows:

1. As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application
2. As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the International application for which fees were paid, specifically claims:
3. No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- The additional search fees were accompanied by applicant's protest.
- No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210 (suppl. sheet (2))

1. Although claims 14-26 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.
2. The expression "inhibiting the advanced glycosylation of a target protein" is not a clear description of a therapeutic application. The search was therefore performed for the use against protein aging and for the amelioration of the complications of diabetes.

Claims searched incompletely: 14-26

3. In view of the large number of compounds which are defined by the general formula of claims 1-3,5,14-16,18, 27 the search was limited to the compounds mentioned in the description page 9 and pages 20-21 and in the claims 4,6-9,11,13,17,19-22,24,26.

Claims searched incompletely: 1-3,5,10,12,14-16,18,23,25,27

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.

US 9200152
SA 56824

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 27/05/92. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A- 9006117	14-06-90	US-A-	4912111	27-03-90
		AU-A-	4626389	26-06-90
		EP-A-	0447414	25-09-91
EP-A- 0330263	30-08-89	JP-A-	1249757	05-10-89
		US-A-	4980350	25-12-90
EP-A- 0316852	24-05-89	US-A-	4908446	13-03-90
		US-A-	4983604	08-01-91
		JP-A-	2000156	05-01-90
		US-A-	4978684	18-12-90
		US-A-	5096703	17-03-92
EP-A- 0222313	20-05-87	US-A-	4758583	19-07-88
		AU-B-	610056	16-05-91
		AU-A-	6495886	21-05-87
		CA-A-	1294218	14-01-92
		JP-A-	3103163	30-04-91
		JP-A-	62142114	25-06-87
		US-A-	4908446	13-03-90
EP-A- 0359112	21-03-90	JP-A-	2167264	27-06-90
EP-A- 0339496	02-11-89	JP-A-	2042053	13-02-90
EP-A- 0327919	16-08-89	US-A-	4978684	18-12-90
		AU-A-	2893589	03-08-89
		JP-A-	2056413	26-02-90
		US-A-	5096703	17-03-92